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Phylogenetic analysis of classical swine fever virus (CSFV) field isolates from outbreaks in South and Central America

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Abstract

To date, there is little information concerning the epidemiological situation of classical swine fever (CSF) in the Americas. Besides summarizing the available data, genotyping of isolates from outbreaks in domestic pigs in several countries of South and Central America was performed. For this, a 190 base fragment of the E2 envelope glycoprotein gene was used. European strains and isolates, and historical isolates from the United States (US) were included for comparison. In contrast to the situation in most parts of Europe, where group 2 isolates predominate, it was found that all the isolates from the American continent analyzed belonged to group 1 and were further resolved into three subgroups. The Cuban isolates clustered in subgroup 1.2, whereas the isolates from Honduras and Guatemala clustered in subgroup 1.3. The remaining isolates from Argentina, Brazil, Colombia and Mexico generated four poorly resolved clusters in subgroup 1.1, together with the vaccine strains, with historical European and US isolates, and with a recent Russian isolate. While the vaccine strains and the historical European isolates formed a relatively distinct cluster, one of the US isolates clustered together with the Mexican, and another one with Colombian isolates. Historically, CSF (hog cholera) was observed almost simultaneously in the US and in Europe in the first half of the 19th century, and its origin remains a matter of discussion. Our results showed that the US isolates are closely related to isolates from South America, while appearance of isolates in Cuba on one hand and in Honduras and Guatemala on the other hand, seems to have been due to unrelated events. This allows to speculate that at least in the American continent, CSF virus may have appeared independently in several regions, and spreading may have been a secondary effect.

Keywords: Classical swine fever; South America; Central America; Genotype; Epidemiology

1. Introduction

Classical swine fever (CSF) virus, a *Flavivirus*, is the causative agent of an economically important disease of pigs world-wide. CSF has been classified as an OIE list A disease and most countries have control and eradication programs for the disease (Paton and Greiser-Wilke, 2003).

There exists relatively little information concerning the situation of CSF in the Americas. The data available for the year 2003 are summarized in Fig. 1 (Anonymous, 2004a). Only Canada and USA are internationally considered to be free of the disease. The disease is present in many other American countries, causing economically devastating outbreaks like in Cuba between 1993 and 1997 (Frias-Lepoureau, 2002)

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Fig. 1. Compilation of the occurrence of CSF in the Americas in 1993 (Anonymous, 2004a): (0000) disease never reported; (NR) disease not reported (date of last outbreak not known); (month/year) date of the last reported occurrence of the disease in previous years; (+) reported present or known to be present; (Z) disease limited to specific zones. The names of the countries appearing in italics and bold are from which isolates were included in this study.

and 2002–2004 (Anonymous, 2004b), and in the Dominican Republic since 1996 (Lubroth, 1999; Teran et al., 2004). On the contrary, there are several countries where the disease has never been reported, and others which did not report to the OIE. The data available for 2004 are still incomplete, yet more than 86 outbreaks of CSF were reported from Cuba, 27 from Peru, and one each from Brazil and Nicaragua, respectively (Anonymous, 2004c).

Remarkable strides have been made in controlling CSF in Mexico (Frias-Lepoureau and Greiser-Wilke, 2002), in Colombia and in certain countries of the Southern cone of South America. Uruguay has not reported cases of CSF since 1991 and stopped vaccination in 1995, whereas Chile has reported the last outbreak in 1996 and forbidden vaccination in 1997. Control programs based on vaccination, laboratory testing, stamping out (depopulation), quarantine, control of transit and import restrictions implemented in Argentina and Brazil reduced the number of outbreaks significantly. The last outbreak in Brazil was reported to the OIE in June 2004, in the State of Ceará (in the north-eastern part of the country) (Anonymous, 2004c). Currently, the Southern states of Brazil and Argentina, being free of the disease, have stopped vaccination against CSF. The Mercosur Trade Alliance, where Argentina, Brazil, Uruguay and Paraguay are in the process of

lifting commercial barriers, has also generated the motivation for improved animal disease control in the region. As a result, efforts towards eradication of CSF are expected to be enhanced, particularly among Mercosur members (Edwards et al., 2000). In addition, the Food and Agriculture Organization of the United Nations (FAO) has implemented the Continental Plan for Classical Swine Fever Eradication in the Americas (http://www.rlc.fao.org/prior/segalim/animal/ppc/peste_porcina/). In a world wide unique combined effort of the participating countries, modern diagnostic methods will be harmonized and molecular techniques implemented in the laboratories, thus enhancing the attempts to eradicate the disease (Teran et al., 2004).

Genotyping has been widely used to assist epidemiological studies. The nucleotide sequences of fragments of the 5′ non-coding region (5′-NTR), of the E2 envelope glycoprotein or the non-structural protein NS5B genes were shown to be useful for classifying CSF virus strains and isolates into groups and subgroups (Björklund et al., 1999; Lowings et al., 1996; Paton et al., 2000). Furthermore, phylogenetic analysis of these sequences provides invaluable information about the possible origin of outbreaks and virus spread in the field. In this study, we have performed phylogenetic analysis of CSF virus isolates originating from the Americas, based on the partial nucleotide sequences of the E2 glycoprotein gene.

2. Materials and methods

2.1. Virus isolates and sequences

The CSFV isolates analyzed in the study are listed in Table 1. They were obtained from the virus collection of the EU Reference Laboratory for CSF (EURL, Germany). Animal and Plant Health Inspection Service (Aphis-USDA, USA), Instituto Colombiano Agropecuario (ICA, Colombia), and Servicio Nacional de Sanidad Animal y Calidad Agroalimentaria (SENASA, Argentina). Additional sequences of American and European isolates were obtained from the GenBank database, or from the CSF database held in the EURL (Greiser-Wilke et al., 2000a).

2.2. RNA extraction, RT-PCR and sequencing

Minced organ material or Infected PK-15 cells were harvested and total RNA was extracted using the TRIzol™ (Gibco BRL) reagent according to the manufacturer's protocol. Reverse transcription (RT) reactions were performed using random primers and the genomic region that encodes the E2 glycoprotein was amplified by PCR. The following primer set (Paton et al., 2000) was used: forward primer 5′-TCR WCA ACC AAY GAG ATA GGG-3′ (positions 2467–2487 from the genome of the Alfort strain) and reverse primer 5′-CAC AGY CCR AAY CCR AAG TCA TC-3′ (positions 2738–2716 in Alfort strain). The PCR products were sequenced using the Cy5 sequencing kit (Amersham Biosciences) on an ALFExpress II automatic sequencing machine (Amersham Biosciences).

2.3. Phylogenetic analyses

The nucleotide sequences were edited, aligned and analyzed as reported previously (Paton et al., 2000). Briefly, the sequences were trimmed to obtain the 190 nucleotide fragments and then aligned using the ClustalX, Version 1.8.3, program (Thompson et al., 1994). The transition/transversion rate was calculated using the TreePuzzle, Version 5.0, program (Strimmer and von Haeseler, 1996, 1997). Boostraping values were calculated with the modules SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package (Felsenstein, 1989). The phylogenetic trees, calculated by using the neighbor-joining method, were computed with the DNADIST and NEIGHBOR modules with the same parameters. For visualization and printing of the trees, the TREEVIEW program, Version 1.6.6 (Page, 1996) was applied. To make the trees comparable to those from other studies, they were outgrouped to the sequence of the Kanagawa isolate. The nomenclature of the subgroups was as previously established (Paton et al., 2000).

3. Results and discussion

In this study, CSF virus isolates from only seven South and Central American countries were included. Unluckily, it was not possible to obtain samples from more countries, due to the fact that organs or virus isolates from CSF cases were not stored for further analyses by the corresponding laboratories.

As was found for several geographical regions in Europe (Biagetti et al., 2001; Hofmann, 1996; Stadejek et al., 1996, 1997), our results indicate that the isolates from the American continent also can be allocated to different geographic regions by genetic typing. While the European isolates (excepting Russian isolates; Vlasova et al. (2003)) all belonged to the different genetic subgroups within group 2, the American isolates analyzed so far belonged to one of the subgroups within group 1 (Fig. 2). This subgroup also includes older European isolates, historical isolates from the US, and all vaccine strains analyzed up to date. Within this group, three subgroups can be distinguished, namely subgroups 1.1, 1.2, and 1.3 (Paton et al., 2000).

In Cuba, CSF was reported for the first time in the decade of the 1940's, and may have been introduced by pigs imported from the USA (Frias-Lepoureau, 2002). The disease was kept under control until 1993 through a National Control Program with vaccination campaigns and a strict epidemiological surveillance. However, in 1993–1997, new CSF outbreaks occurred (Anonymous, 1997; Frias-Lepoureau, 2002), coupled with a worsening economic situation on the country. The Cuban isolates from these outbreaks clustered in subgroup 1.2, forming an Eastern and a Western cluster, respectively

Table 1
Origin of the CSF virus isolates included in this study

Code[a]	Place of origin	Isolate name	Year	Accession number[b]	Reference
Subgroup 1.1: Argentina					
XXX0115	Argentina	UBA3	?	AJ781108	This work
XXX0116	Argentina	Casilda	1978	AJ781109	This work
XXX0117	Argentina	DS	?	AJ781110	This work
Subgroup 1.1: Colombia (2); Mexico; Brazil (2); European vaccines, historical US isolates; Russian isolate					
XXX0055	Colombia	Santander	1980	AY308857	This work
XXX0101*[1]	Colombia	Zulia	2003	AY535802	This work
XXX0102*[1]	Colombia	Cucuta	2003	AY535803	This work
XXX0103*[1]	Colombia	Cicarsia	2003	AY533485	This work
XXX0105	Colombia	Choachi	2003	AY534987	This work
XXX0050	Colombia	5704-Pte.Nacional	2002	AY308862	This work
XXX0046*[2]	Colombia	7609-Boyaca	2002	AY308866	This work
XXX0048*[2]	Colombia	7487-Velez	2002	AY308864	This work
XXX0045*[2]	Colombia	8281	2002	AY308867	This work
XXX0047*[3]	Colombia	7510-Ibague	2002	AY308865	This work
XXX0049*[3]	Colombia	6588-Ibague	2002	AY308863	This work
XXX0051*[3]	Colombia	5985-San Andres	2002	AY308861	This work
XXX0056*[3]	Colombia	4755-La Calera	2002	AY308856	This work
XXX0057*[3]	Colombia	4650-Zipaquira	2002	AY308855	This work
XXX0059*[3]	Colombia	4191-Tequendama	2002	AY308853	This work
XXX0060*[3]	Colombia	38-47-Tena	2002	AY308852	This work
XXX0061*[3]	Colombia	3812-Zipaquira	2002	AY308851	This work
XXX0062*[3]	Colombia	3764-Zipaquira	2002	AY308850	This work
XXX0099*[3]	Colombia	Calarca	2003	AY535800	This work
XXX0100*[3]	Colombia	Coello	2003	AY535801	This work
CSF0656	Mexico	105	1992	AJ781107	This work
CSF0645	Mexico	T97006	1997	AJ781106	This work
CSF0647*[4]	Mexico	31719#1	1997	AJ781104	This work
CSF0649*[4]	Mexico	31719#4	1997	AJ781105	This work
CSF0655	Mexico	1121	1991	AJ781103	This work
CSF0654	Mexico	91–1090	1991	AJ781102	This work
CSF0775	Brazil	09/Baco	1995	AJ781101	This work
CSF0766	Brazil	22497	?	AJ781100	This work
CSF0913	Germany	Riems vaccine	?		EURL
CSF0908	China	C- strain (vaccine)	?		This work
XXX0137	United States	Fort Dodge	1954		Lowings et al., 1996
XXX0136	United States	Old Lederle	1946		This work
CSF0918*[5]	United States	Baker 331	1969		This work
CSF0696	Russia	762/Ru	1999		Vlasova et al., 2003
CSF0911*[5]	Germany	Glentorf	1968		Lowings et al., 1996
CSF0902	France	Alfort 187	1987		This work
Subgroup 1.1: Brazil (1); Colombia (1)					
CSF0307*[6]	Brazil	EVI192	?		Paton et al., 2000
XXX0001*[6]	Brazil	EVi100	1987		This work
XXX0052	Colombia	3223-Zipaquira	1998	AY308860	This work
XXX0053	Colombia	2094-Pto. Asis	1999	AY308859	This work
XXX0054	Colombia	1831-Mocoa	1999	AY308858	This work
Subgroup 1.2: Cuba, Europe, US					
XXX0120	Cuba	33/97E	1997		Diaz de Arce et al., 1999
XXX0121	Cuba	253/96E	1996		This work
XXX0118*[7]	Cuba	Margarita	1958		This work
XXX0122*[7]	Cuba	Camacho/93	1993		This work
XXX0119	Cuba	15/97E	1997		This work
CSF0705	Cuba	39 (vaccine)	?	AJ781111	This work
CSF0932	United States	Baker A	?		Lowings et al., 1996
CSF0929	Italy	Brescia	1945	M31768	Moormann et al., 1990
Subgroup 1.3: Honduras; Guatemala					
XXX0113	Honduras	Honduras92	1992		Paton et al., 2000
CSF0653	Honduras	HCV31	1992	AJ781098	This work
CSF0644	Honduras	9346	1996	AJ781097	This work
CSF0643	Honduras	7446	1996	AJ781096	This work

Table 1 (*Continued*)

Code[a]	Place of origin	Isolate name	Year	Accession number[b]	Reference
CSF0651	Guatemala	5502	?	AJ781095	This work
CSF0650	Guatemala	4409	?	AY672637	This work
Group 2: European isolates					
CSF0001	Germany	2699/Osterode	1982		EURL
CSF0296	Poland	31/2	1992		EURL
CSF0283	Netherlands	MP104	1997		Greiser-Wilke et al., 2000b
CSF0022	Germany	907/2	1989		EURL
CSF0115	Austria	SP7980	1992		Bartak and Greiser-Wilke, 2000
CSF0404	Italy	5103VA/97	1997		EURL

The *^{n} denotes the isolates with identical sequences in the region sequenced; n = 1–7.

[a] CSFV sequence database code (Greiser-Wilke et al., 2000a,b).

[b] GenBank accession number; (?) year of isolation is not known.

(Diaz de Arce et al., 1999), and there was no relationship to other South or Central American isolates analyzed so far (Fig. 2). This may be due to the facts that Cuba is an island, and that commercial trade is largely blocked, which minimizes contacts with other countries. Thus, it is not likely that CSF was due to an external reintroduction (Frias-Lepoureau and Greiser-Wilke, 2002). Also, the US isolate (CSF0932; Baker A) and the Italian strain Brescia (CSF0929), which also belong to subgroup 1.2, formed a clearly separated cluster (Fig. 2).

The isolates from Guatemala and Honduras were in a different subgroup (1.3). The isolates from Guatemala were closely related to the isolates from Honduras from 1996; the remaining Honduran isolates from 1992 seem to be clearly different (Fig. 2). Whether the outbreaks in the first cluster were related cannot be determined, as epidemiological data are lacking. On the other hand, it is conceivable that the virus was transferred from one of these two neighboring countries to the other by trade.

The South American and Mexican isolates analyzed formed four clusters in subgroup 1.1 (Fig. 2). In one cluster, isolates from Brazil (1987) and from outbreaks in 1998/99 in Colombia were distinguishable. More recent Colombian isolates from outbreaks in 2002–2003 were clearly separated from the first cluster, indicating that introduction of a new CSF isolate had occurred. The third cluster contained the remaining isolates from Mexico and more recent (1995) isolates from Brazil, but individual clusters were supported by low bootstrap values only. The isolates in this cluster are closely related to vaccine strains like the attenuated China strain, derivatives of which are widely used for vaccination throughout the world. Albeit with low resolution, the isolates from Argentina formed a fourth cluster.

The outbreaks in Colombia in 1999 occurred in the department of Putumayo in the south of the country; the commercial activity in this area (border to Peru and Brazil) may have led to the appearance of this virus. On the other hand, in the previous year isolates of the same subgroup had occurred in the center of the country (Zipaquirá – Cundinamarca). The outbreaks in 2002 and 2003 were caused by isolates belonging to a different cluster within subgroup 1.1 (Fig. 2). The outbreaks extended from the center of the country (Cundinamarca) towards the departments of Boyacá, Tolima, Quindío, North of Santander and to the island of San Andrés, but not to other islands in the Caribbean.

Brazil implemented an eradication program in 1992. The program was designed to achieve eradication in a progressive way, starting in areas where the swine production is more intense. The country was divided into three areas: (1) the Southern states, which are free of the disease without vaccination; (2) states with a relatively large swine population where CSF was still endemic and vaccination was made compulsory; (3) the rest of the country where swine production is not significant and vaccination was not made compulsory. Today, the states of Rio Grande do Sul, Santa Catarina, Paraná, Minas Gerais, and Mato Grosso do Sul have been declared free of CSF (Frias-Lepoureau and Greiser-Wilke, 2002). The epidemiological data available for the Brazilian isolates is incomplete, as there is no information whether they originated in the North or in the South of the country. Therefore the only conclusion that can be drawn from these results is that the older outbreaks (around 1987) and those from around 1995 are due to different events.

Mexico has three distinct areas: (1) the Northern states on the border with USA plus the Yucatan peninsula are free of CSF without vaccination; (2) the Central part has the status of an eradication area where vaccination has been prohibited; (3) the Southern part of the country has the status of control area, where the disease is endemic and vaccination is used continuously (Frias-Lepoureau and Greiser-Wilke, 2002). No outbreaks were reported to the OIE in 2004, but there were six outbreaks in 2002 and 2003, respectively (Anonymous, 2004a, 2004c). Unluckily, no isolates from these outbreaks were available.

Our results allow the conclusion that in the Americas at least three unrelated events led to the appearance of CSF virus. In addition, inclusion of four historical isolates from outbreaks in the US encourages speculation concerning the origins of the CSF virus. No exact data exist, but a report of a USDA Bureau of Animal Industry from 1887–1888 indicates that the disease (then named hog cholera) had been noted first in Ohio, USA in 1833 (Liess, 1981). Other reports suggest that also in Europe it must already have been

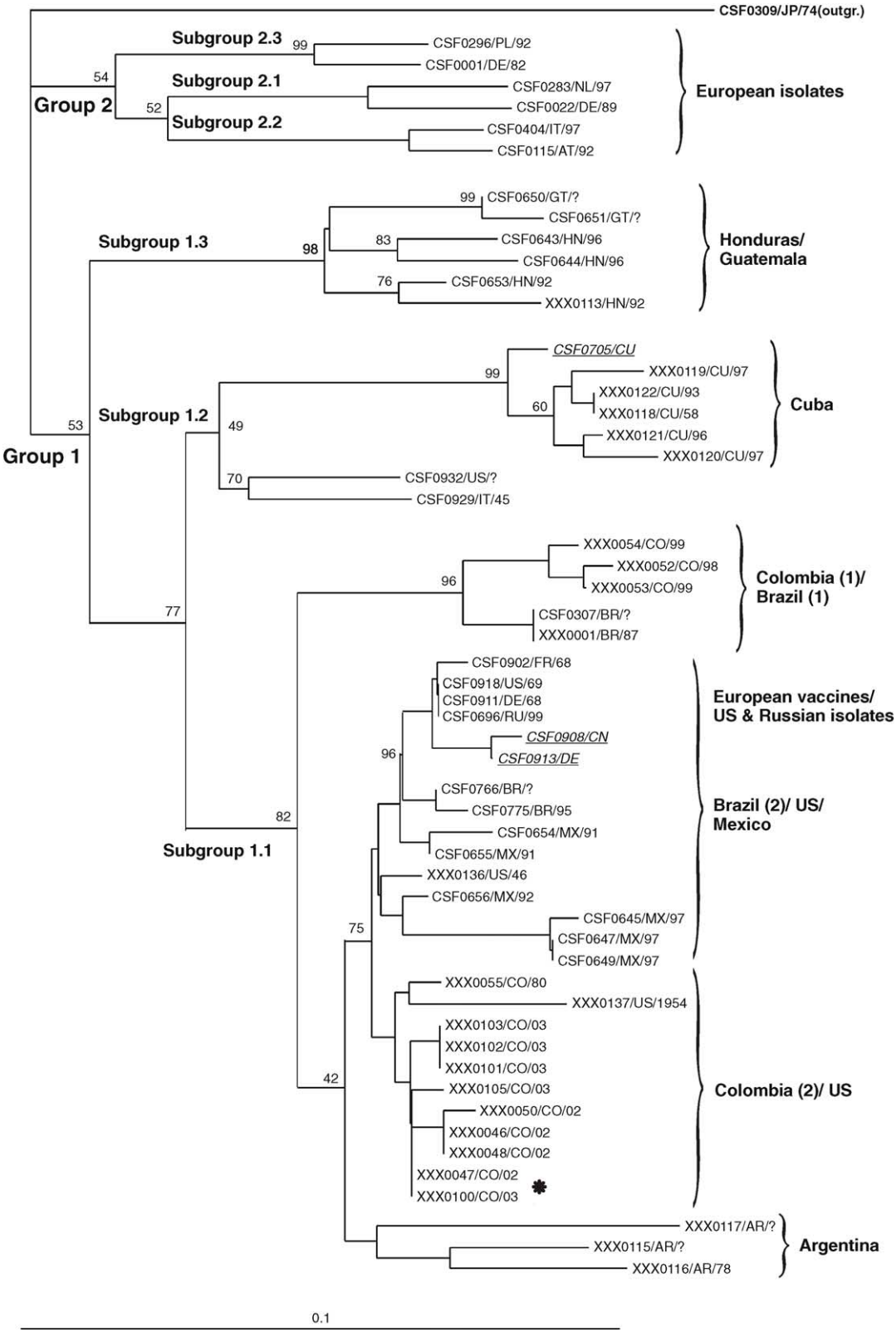

Fig. 2. Neighbor-joining phylogenetic tree constructed from a 190 nucleotide fragment of the E2 gene. The tree was outgrouped to the sequence of the Kanagawa isolate (CSF0309). The isolates are named by their code (Table 1). Group and subgroup nomenclature is as proposed by Paton et al. (2000). Vaccine strains are in italics and underlined; '*' denotes group of 11 isolates with identical sequences in the 190 b region analyzed (Table 1); bar: number of substitutions per site.

present in the first part of the 19th century (Beynon, 1962). Each of the US isolates included in this study clustered with isolates from different parts of the Americas. This suggests that CSF viruses may have originated simultaneously almost worldwide, including in Asia, as up to date only here group 3 CSF virus isolates have been found (Paton et al., 2000). Then, some isolates may have spread – probably by trade – to several other locations in the world.

Although several clusters of CSF viruses that are characteristic for the geographical regions and for specific outbreaks are clearly evident, the missing epidemiological data and the restricted amount of available isolates greatly hampers the interpretation of the results. Nevertheless, it is noteworthy that some countries on the American continent seem to be isolated enough to harbor own characteristic CSF virus isolates. This is evident for Cuba, but seems to be the case for Guatemala and Honduras in Central America. For obtaining a clearer picture, a closer cooperation between the veterinary authorities and diagnostic laboratories working on CSF eradication will be necessary. In the context of the Continental Plan for Classical Swine Fever Eradication in the Americas (http://www.rlc.fao.org/prior/segalim/animal/ppc/peste_porcina/), it is planned to create a regional database containing the sequences of new American isolates and the corresponding epidemiological data.

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